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Immunology

Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans

(autoimmunity/cross-reactivity/adjuvant arthritis)

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ABSTRACT **Adjuvant arthritis characterized by chronic inflammation of the joints of rats is induced by immunization to *Mycobacterium tuberculosis*. To learn how autoimmune arthritis may be caused by a microbial antigen, we isolated a T-lymphocyte clone specific for *M. tuberculosis* antigens that was strongly arthritogenic. We now report that the clone recognized, in addition to *M. tuberculosis* antigens, antigens present in human synovial fluid, medium of chondrocyte cultures, and proteoglycans purified from cartilage. These observations indicate that the target antigen for the arthritogenic clone resides in the proteoglycan component of cartilage. As this arthritogenic clone shows specificity for both a *M. tuberculosis* antigen and a cartilage constituent we conclude that disease is probably caused by antigenic cross-reactivity. Thus, an autoimmune disease may be triggered by structural mimicry between antigens in the environment and self-antigens in the individual.**

Adjuvant arthritis can be produced in genetically susceptible rats by inoculation of *Mycobacterium tuberculosis* emulsified in oil (complete Freund's adjuvant) (1). The disease has been thought to involve an autoimmune process because it can be transferred by lymphocytes from affected to healthy rats (2). Adjuvant arthritis therefore poses the question of how an autoimmune disease is triggered by immunization to microbial antigens. Our strategy for investigating autoimmunity has been to isolate and grow as long-term cell lines specifically autoimmune T lymphocytes from animals with experimentally induced diseases such as experimental autoimmune encephalomyelitis (3) or experimental autoimmune thyroiditis (4). These cell lines are functional and can mediate the specific disease *in vivo* or under suitable conditions can be used as vaccines to endow recipient animals with resistance to active induction of the particular disease (4–6).

The development of functional T-lymphocyte lines in the experimental autoimmune encephalomyelitis and experimental autoimmune thyroiditis models was guided by knowledge of the target self-antigens, myelin basic protein and thyroglobulin, that can be used to select the lymphocytes *in vitro*. In adjuvant arthritis, in contrast, the putative target self-antigen was unknown. Nevertheless, an arthritogenic line, designated A2, was raised from lymph nodes of affected rats using ground *M. tuberculosis* as the selecting antigens (7). We reasoned that line A2 was arthritogenic because some of its cells had receptors specific for *M. tuberculosis* antigens that possibly mimicked self-antigens in the joints.

Subsequent cloning of the A2 T-cell line provided us with a T-lymphocyte clone that was both immune to *M. tuberculosis* and also arthritogenic (8). This clone, A2b, was exploited in the present investigation as a probe to detect the presumed target self-antigen by measuring the *in vitro* proliferative responses of the clone and also its *in vivo* delayed type hypersensitivity reactivity to various joint substances.

METHODS

Rats. Inbred Lewis rats, 2–4 months old, were obtained from the Animal Breeding Center of this Institute. Rats were matched for age and sex in each experiment.

Clone A2b. The isolation, cloning, and maintenance of the T-lymphocyte clone A2b has been described previously (8). Briefly, a line reactive to *M. tuberculosis* was first isolated from draining lymph nodes of Lewis rats immunized with complete Freund's adjuvant (7). The established line was cloned by seeding a dilution of 0.1 cell per well (96-well tissue culture plate, Costar, Cambridge, MA) in the presence of irradiated (1500 rads; 1 rad = 0.01 gray) syngeneic thymocytes (2×10^7/ml) and *M. tuberculosis* (10 μg/ml). Cloned cells were expanded in interleukin 2-containing propagation medium. Every 2–4 weeks clone A2b was restimulated by incubation with *M. tuberculosis* and accessory cells for 3 days and then transferred back into propagation medium as described (8).

Response to Antigens. The cloned cells were incubated in flat-bottomed microtiter plates in triplicate wells (3, 7, 8). Each well contained 2×10^4 cloned cells and 2×10^6 1500-rad–irradiated syngeneic thymocytes in 0.2 ml of proliferation medium (Dulbecco's modified Eagle's medium supplemented with 1% syngeneic rat serum) with antigens in various concentrations. After 24 hr of incubation, 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine (specific activity, 10 Ci/mmol; Nuclear Research, Negev, Israel) was added to each well and incubation was continued for 18 hr. Cultures were harvested on fiberglass filters and thymidine incorporation was measured using a liquid scintillation counter. Thymidine incorporation is expressed as mean cpm of test cultures minus mean cpm of control cultures without antigen ± standard deviation (Δcpm).

Antigens. The following antigens were used: heat-killed *M. tuberculosis* $H_{37}Ra$ purchased from Difco; proteoglycan extracted as described by Haskall and Kimura (9) from rat and pig cartilage by G. Hunter (kindly donated by A. Czitrom, Mount Sinai Hospital, Toronto); conditioned medium of chicken chondrocyte culture harvested from a 12-day-old suspension culture of chondrocytes (5×10^5 cells per ml) in soft agar (10); cell-free synovial fluid obtained by puncture from a human osteoarthritic joint; collagen type II derived from rat cartilage and keratan sulfate derived from human cornea (kindly donated by E. J. Miller of the Alabama Medical Center, Birmingham, and T. Laurent of the Univer-

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sity of Uppsala, respectively); chondroitin sulfate (purity grade III) and hyaluronic acid (purity grade IV) purchased from Sigma; and core protein of cartilage proteoglycan prepared as described (11).

Delayed Type Hypersensitivity. Delayed type hypersensitivity induced by clone A2b was measured as follows. Clone A2b was activated by restimulation in proliferation medium in the presence of 10 μg of *M. tuberculosis* antigen/ml and irradiated syngeneic thymocytes as accessory cells (3, 7, 8). Cells (2×10^7) were injected intravenously into groups of five irradiated (750 R) or intact Lewis rats. To test for delayed type hypersensitivity, 5 days later 0.1 ml of each antigen solution was injected with a 27-gauge needle into the pinna of a rat's ear at a concentration of 200 μg/ml for *M. tuberculosis* and chondroitin sulfate and at a dilution of 1:20 for synovial fluid. Ear thickness was measured with a micrometer caliper (Ames, Waltham, MA) at 24 hr and at 48 hr after injection of the antigen. No significant differences were observed between measurements at 24 and 48 hr and the results are shown at 24 hr. The results are expressed as mean percent increase of ear thickness (mean ear thickness 24 hr after injection − mean ear thickness before injection) ± SD. Delayed type hypersensitivity reactions were similarly measured in rats in which active adjuvant arthritis had been induced as described (7, 8) 4 weeks earlier.

RESULTS

As shown in Fig. 1, the cells of clone A2b responded *in vitro* ([^{3}H]thymidine incorporation) to synovial fluids isolated from humans suffering from osteoarthritis, to conditioned medium of chicken chondrocytes grown in suspension culture, and to preparations of rat or porcine proteoglycans. Similar results were obtained using synovial fluids from patients with rheumatoid arthritis (data not shown). These responses were considerable, although the [^{3}H]thymidine incorporation of clone A2b stimulated by *M. tuberculosis* exceeded them by an order of magnitude. Responses to collagen type II, the predominant collagen constituent of cartilage, and to the glycosaminoglycan constituents of cartilage, hyaluronic acid and keratan sulfate, were invariably found to be negative. The lack of responsiveness to collagen type II clearly distinguishes this model from collagen II arthritis (12). The positive responses of clone A2b to proteoglycan-containing preparations such as synovial fluid and chondrocyte medium and to purified proteoglycans were specific for that clone and were not due to contamination of the antigens with some nonspecific mitogen. No response to these preparations was shown by two other clones, a non-arthritogenic but *M. tuberculosis*-specific clone that had been isolated from the same line as had clone A2b and an encephalitogenic, basic protein specific T-lymphocyte clone (results not shown).

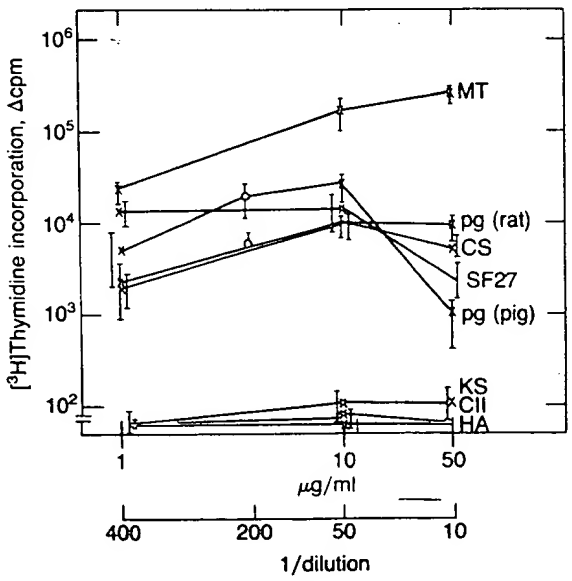

FIG. 1. *In vitro* [^{3}H]thymidine incorporation by the arthritogenic T-lymphocyte clone A2b after stimulation by *M. tuberculosis* (MT) and various cartilage components. pg, Proteoglycan preparation of rat or pig cartilage; CS, conditioned medium of chicken chondrocyte culture; SF27, cell-free synovial fluid from a human osteoarthritic knee.

It has been difficult to identify the particular epitope which the cells of clone A2b recognized in synovial fluid and proteoglycan preparations. For example, the cells of clone A2b showed [^{3}H]thymidine incorporation in two of four experiments when incubated *in vitro* with a preparation containing chondroitin sulfate (results not shown). The lack of uniformity in the response to chondroitin sulfate might be explained by the observation that carbohydrate antigens often are not taken up and processed efficiently by antigen-presenting cells *in vitro* (13).

We observed previously that T-cell lines transferred antigen-specific delayed type hypersensitivity skin reactions and that irradiation of the recipient rats enhanced the magnitude of transferred reactivity (14). Clone A2b which bears the W3/25 marker of helper/delayed hypersensitivity T lymphocytes (8) was tested for its ability to transfer delayed type hypersensitivity to *M. tuberculosis* or to the cross-reactive determinant present in joint materials. Fig. 2 shows the results of delayed type hypersensitivity reactivities as measured by ear swelling 24 hr after intrapinnal injection of antigen into rats, some of which had been irradiated and/or had received clone A2b. It can be seen that in irradiated recipients clone A2b transferred delayed type hypersensitivity reactivity not only to *M. tuberculosis* but also to antigens present in synovial fluid and to a chondroitin sulfate preparation of proteoglycan. A delayed hypersensitivity response was also elicited by a core protein of proteoglycan prepared by exhaustive digestion of the Swarm rat chondrosarcoma proteoglycan by hyaluronidase (11) (results not shown). Histologic examination of the ear swelling confirmed that this response was caused by a delayed type hypersensitivity reaction.

A delayed type hypersensitivity reaction was elicited in the nonirradiated recipients of clone A2b by *M. tuberculosis* but not by chondroitin sulfate or synovial fluid, an observation for which there is no obvious explanation. It is conceivable that radio-sensitive regulatory mechanisms control responsiveness to proteoglycan self-determinants, a hypothesis supported by the finding that irradiation of the recipient is a prerequisite for induction of arthritis by line A2 or clone A2b (7, 8). However, irradiation of the recipient was not required for other line-mediated autoimmune diseases such as experimental autoimmune encephalitis in rats (3) or experimental autoimmune thyroiditis in mice (4).

Reactivity to proteoglycans was also observed in intact rats suffering from active adjuvant arthritis. Table 1 shows the delayed type hypersensitivity reactivities of rats that had been inoculated with complete Freund's adjuvant 4 weeks earlier to induce arthritis. Similar to the irradiated recipients of clone A2b, these unirradiated rats responded to *M. tuberculosis* and in addition to synovial fluid and to the chondroitin sulfate and core protein components of proteoglycan. They did not respond to the keratan sulfate chains of proteoglycan. Thus, the proteoglycan cross-reactivities demonstrated by the arthritogenic clone A2b were confirmed in unirradiated rats with actively induced disease.

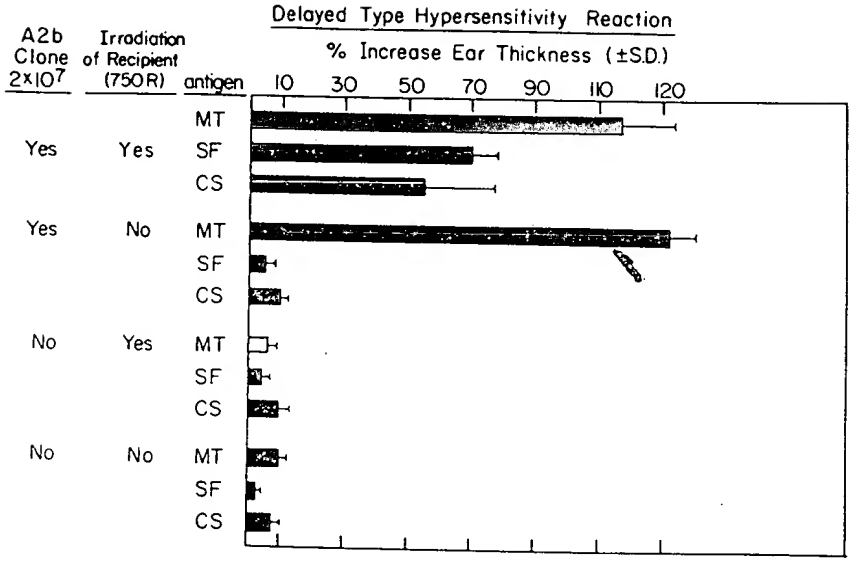

FIG. 2. Delayed hypersensitivity ear test. The % increase in ear thickness was measured 24 hr after injection of antigens; MT, *M. tuberculosis*; SF, human synovial fluid; CS, chondroitin sulfate.

DISCUSSION

The results of this study demonstrate that a T-cell clone selected by the strength of its reactivity to mycobacterial antigens also recognized part(s) of a proteoglycan molecule of cartilage, possibly the core protein or the chondroitin sulfate chain. A more precise identification of the epitope thus far has been hampered by the paucity of techniques suitable for isolation of undenatured fragments of proteoglycans. In general, responses to core protein have tended to be stronger than those observed to chondroitin sulfate and it is possible that the responses to chondroitin sulfate preparations may have been due to contamination with core protein.

As a monoclonal population of lymphocytes should deploy antigen receptors of a uniform shape, the sensitivity of the cells of clone A2b to both *M. tuberculosis* and proteoglycans argues for the existence of structural mimicry between their parts. The differences in magnitude of [^{3}H]thymidine incorporation of clone A2b in response to *M. tuberculosis* and to proteoglycan materials suggests that their mimicry may be imperfect or that the self-epitope might be only a minor component of the proteoglycan preparations that were available to us.

Cross-reactivity between microbial antigens and self-molecules has been seen previously by means of serology. Group A streptococci were shown to cross-react with human myocardium, a mimicry that might play a role in the pathogenesis of rheumatic fever (15). Monoclonal antibodies to DNA raised from the lymphocytes of patients with lupus erythematosus bind cardiolipin and other substances and it has been proposed that DNA-binding antibodies in these patients may have arisen in response to cross-reactive antigens introduced by bacteria (16). Worthy of note is the association of some types of arthritis with previous infection of humans of *HLA-B27* genotype with enteric bacteria (17).

The demonstration of a serological cross-reactivity, although intriguing, does not prove its role in pathogenesis. We have now shown in the present investigation that the arthritogenic clone A2b itself is the very agent that defines a structural mimicry between *M. tuberculosis* and joint cartilage. Hence, this cross-recognition indeed may incite the immune attack on the joints characteristic of adjuvant arthritis. Moreover, the pathogenic consequences of antigenic mimicry between mycobacteria and joint cartilage may not be limited to rats; a significant number of persons treated for cancer by repeated immunization with the Bacillus Calmette–Guerin strain of mycobacteria have developed arthritis (18).

Table 1. Delayed type hypersensitivity response of rats with active adjuvant arthritis

Antigen	% increase in ear thickness
M. tuberculosis	86 ± 7
Synovial fluid	33 ± 14
Core protein	48 ± 20
Chondroitin sulfate	16 ± 8
Keratan sulfate	0 ± 2

Groups of five Lewis rats were inoculated with complete Freund's adjuvant to induce active adjuvant arthritis (7, 8). Four weeks later delayed hypersensitivity responses were assayed. The background swelling induced by the antigens in normal rats was subtracted from the mean response. Results represent mean ± SD.

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